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(54) METHOD OF DETECTING 2-METHYLISOBORNEOL

(57)Abstract:

PROBLEM TO BE SOLVED: To easily detect 2-MIB with high sensitivity in a short time by utilizing a camphanol having a structure similar to 2-methylisoborneol(2-MIB) which is a mold odor causing material.

SOLUTION: A 2-MIB detecting sensor 1 having a gold electrode mounted on a quartz oscillator to immobilize a camphanol. OVa composite antibody, and the non-covered part of the gold electrode blocked by bovine serum albumin (BSA) is connected to an oscillating circuit 2, a frequency counter 3, and a personal computer 4, and when 2-MIB is adhered to the camphanol. OVa composite antigen, the weight can be measured as the change of frequency. The sensor 1 is set to a measuring cell having a capacity of 150mL, and air passed through activated charcoal 6 in order to a fixed measuring environment is sent in 120mL /minute by an air pump. A vessel of about 500μ l is prepared for dipping the quartz oscillator having the gold electrode, the camphanol. OVa composite solution having a concentration of 0.5mg/mL is prepared, and the camphanol. OVa composite is immobilized on the gold electrode.

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CLAIMS

[Claim(s)]

[Claim 1] The detection approach of 2-MIB using the antigen-antibody reaction of the camphor by which structure was similar to 2-methyl isoborneol (following 2-MIB and publication), the anti-2-MIB antibody produced using proteinic complex, and a 2-MIB of the measuring object, and camphor and a protein complex antigen.

[Claim 2] 2-MIB of the strange concentration which carries out a dipping into the measured solution which prepared the transducer which fixed the camphor and the protein complex antigen with which structure was similar to 2-MIB so that an anti-2-MIB antibody might serve as fixed concentration, and is contained in a measured solution, By output change of the transducer by the anti-2-MIB antibody which the camphor and the protein complex antigen fixed on the transducer were made to react with an anti-2-MIB antibody competitively, and was combined with camphor and a protein complex antigen as a result The detection approach of 2-MIB which carries out the quantum of the 2-MIB concentration in a measured solution.

[Claim 3] An anti-2-MIB antibody given in claims 1 and 2 is the detection

approach of 2-MIB characterized by producing using the complex of camphor and cow serum albumin.

[Claim 4] Camphor and a protein complex antigen given in claims 1 and 2 are the detection approach of 2-MIB characterized by protein being ovalbumin.

[Claim 5] a transducer according to claim 2 -- a quartz resonator or surface acoustic element (SAW component) it is -- the detection approach of 2-MIB characterized by things.

[Translation done.]

* NOTICES *

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the approach an immunoreaction detects 2-MIB which is the mold odor matter contained underwater.

[0002]

[Description of the Prior Art] The complaint about the odor and taste of a waterworks and damage are in the inclination of an increment every year. Mold odor matter with which this odor and taste is generated by extensive generating of the algae by eutrophication of contamination of a source, a lake, etc., such as 2-methyl isoborneol (2-metylisoborneol:2-MIB) and JIEOSUMIN, is the causes.

For this reason, in order that the requests to supply of safe and delicious water may mount among people and may aim at adequate supply of more high quality tap water in response to this request in recent years to tap water, the "comfortable Water-Quality-Standards item" which contains the mold odor matter as an item which complements a drinking water standard is set up. For this criteria achievement, development of the sensor in connection with a smell and an altitude water purification processing system is important.

[0003] Now, in water quality management of water purification, human being is supervising by smelling a smell for every fixed time amount through the nose. Moreover, as an analysis means of a mold odor, purge trap gas chromatography-mass spectrometry and the solid phase extract GC/MS method are adopted as a regulating method. However, these analysis means have some troubles from a viewpoint of continuous monitoring of water quality, it is simple and quick and development of a measuring method and equipment without individual difference is demanded.

[0004] On the other hand, in the quality control field of food stuff industry or chemicals, the "smell sensor" which has a threshold near human being's nose as a measuring instrument about a smell is developed, and it is marketed from several companies. There is a sensor using the lipid covering quartz resonator which detects the smell matter by the metal oxide semiconductor sensor detected by 1 resistance value change, KONDA cutin GUPORIMA - which detects by 2 conductivity change, and 3 weight change etc. in the typical measurement principle of goods.

[0005] Moreover, in a researches-and-developments phase, although assay with the luminescence gene of the mold odor matter etc. is reported (1303 the collection of the 50th time annual academic lecture meeting lecture [besides Yoshinobu Ishibashi] outlines of Japan Society of Civil Engineers, p1302- 1995), the least concentration of 2-MIB in which a quantum is possible is 0.1 mg/L.

[0006]

[Problem(s) to be Solved by the Invention] Although the above-mentioned

regulating method can perform highly precise measurement, in order that it may require skill for three actuation that 1 time of the measuring time is as long as about 5 hours being complicated, and analyzing correctly also including the concentration of two samples with 1 expensive equipment, there are troubles, like there is a difficult field in routine analysis.

[0007] 2 [moreover,] which lacks in functionality with the feeling of the lack of 1 sensibility, or human being about a commercial smell sensor -- in order to distinguish a smell by the pattern comparison of several sorts of sensor responses to a certain smell, 3 quantum measurement which there is no selectivity over a specific smell and needs the analysis of the measurement data based on statistics processing is difficult. Therefore, in order to apply a commercial smell sensor to the odorant monitor of water purification, there are problems, like there is the need of solving problems, such as sensibility, selectivity, and quantum nature.

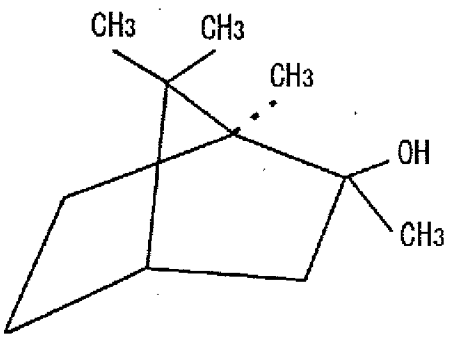
[0008] This invention solves the above-mentioned trouble and aims at offering the measuring method which can detect 2-MIB to high sensitivity for a short time easily.

[0009]

[Means for Solving the Problem] In order to attain this purpose, in this invention, the camphor (Camphor) which has structure similar to 2-MIB which is a mold odor causative agent is used, and an antigen-antibody reaction attains singularity (high selectivity) and high sensitivity.

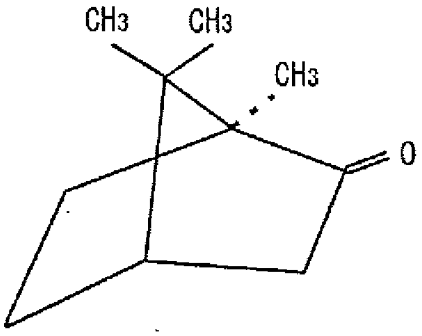
[0010]

[Formula 1]



[0011]

[Formula 2]



[0012] The camphor which specifically has structure similar to 2-MIB which is the above-mentioned mold odor causative agent, and the transducer which fixed the proteinic complex antigen The dipping of the anti-2-MIB antibody to combine is carried out into the solution measured [fixed concentration ****]. 2-MIB -- being specific (alternative) -- 2-MIB of the strange concentration which exists in a solution, and this camphor and protein complex antigen are made to react competitively. As difference from the amount of joint antibodies when the amount of anti-2-MIB antibodies combined with the camphor and the protein complex antigen fixed on the transducer as a result is calculated by output change of a transducer and 2-MIB exists It is characterized by carrying out the quantum of the 2-MIB concentration in a measured solution.

[0013] Moreover, in producing an anti-2-MIB antibody, this invention is characterized by using the complex (following camphor and BSA complex, and publication) of camphor and cow serum albumin (Bovine Serum Albumin: Following BSA and publication) as an antigen. therefore, 2 camphor to which production of an antibody becomes easy by using the camphor of instead of water solubility for 2-MIB which it is refractory in 1 water and is hard to treat in it is low-molecular, and if independent, in order not to show antigenic, the advantage which acquires as it becomes possible to give antigenic, and is said by making it combine with BSA arises.

[0014] Moreover, when camphor and ovalbumin (Ovalbumin: Following Ova and publication) carry out the complex (following camphor and Ova complex, and publication) of the complex used for the competitive reaction of 2-MIB, in the case of a reaction with camphor and Ova complex, the anti-2-MIB antibody which produced camphor and BSA complex as an antigen can recognize only camphor, and can join together.

[0015] Moreover, this invention uses a quartz resonator or a surface acoustic element (SAW component) for a transducer, detects the amount of association of an anti-2-MIB antibody as weight change from frequency-ization before and behind a reaction, and can carry out the quantum of the 2-MIB by the above-mentioned approach.

[0016]

[Embodiment of the Invention]

below the [example 1] and this invention -- a transducer -- a quartz resonator (an AT cut --) Production of the camphor and the Ova complex fixed in ** transducer about the case of 10MHz, ** Production of the anti-2-MIB antibody put in into a measured solution, the singularity trial of a ** anti-2-MIB antibody, and a result, ** Explain in order of production of the detection equipment of quartz-resonator use 2-MIB, and the sensor for 2-MIB detection, the method of detecting 2-MIB using ** competitive reaction, and creation ** of the calibration curve by 2-MIB of ** known concentration.

[0017] ** . -- production [of the camphor and the Ova complex fixed in a transducer]: -- the ethanol of 14mL(s) and the sodium hydroxide of 2 conventions of 16.5mL(s) were added to camphor 1g and 2.84g of orthochromatic carboxy methoxyl amine hemi (O-Carboxymethoxylamine hemi) hydrochlorides, and it flowed back for 6 hours, and was left at 25 degrees C overnight. The pure water of 200mL(s) is added to this, and it is 2 mol/m3. It prepared to pH9.5 using the sodium hydroxide, and this was prepared to pH3 with the hydrochloric acid of 1 convention of a water layer after the 3 times extract with the ethyl acetate of 30mL(s). The camphor carboxymethyl oxime

(Camphor-carboxy methyloxime, Following CMO and a publication) was obtained because put this at 0 degree C overnight, and collect the produced precipitate according to centrifugal separation (it is 5 minutes at 3000 revolutions per minute) and it carries out reduced pressure drying under existence of a calcium sulfate after washing with the pure water of 500mL.

[0018] Next, the tetrahydrofuran (tetrahydrofuran) of 1mL was added to the triethylamine (Triethylamine) of 18.9mg CMO and 12microL, and after cooling and the isobutyl chloro FORU mate (Isobutyl chloroformate) of 12microL were added at -5 degrees C, and it shook for 30 minutes at -5 degrees C. It was dropped into Ova solution 5mL of 20 mg/mL which cooled this reaction mixture, and shook at 4 degrees C overnight. This was dialyzed with pure water and camphor and Ova complex were obtained. Preservation performed cryopreservation at -20 degrees C.

[0019] ** . -- production [of the anti-2-MIB antibody put in into a measured solution]: -- 2-MIB as an antigen was refractory in water, and since handling was difficult, it used for production of an antibody the camphor which has structure similar to 2-MIB. However, since camphor does not have antigenic with low molecular weight, it needs to combine macromolecules, such as protein, and to consider as an antigen. Antigenic weak BSA is comparatively more suitable than Ova which has antigenic [strong] as this protein. Then, camphor and a BSA complex antigen were produced by the same approach as the camphor and the Ova complex stated to **.

[0020] Next, this antigen was diluted with PBS (Phosphate Buffered Saline) for production of an anti-2-MIB antibody, and the emulsion for immunity was produced using 300microL of 300micro of this diluent L, and the Freud perfect AJU band (Freund Complete Ajuvant) by "Nakarai Tesuku, Inc." Intraperitoneal injection was performed to the "Narikazu laboratory animal" BALB/C mouse of 5 weeks-old male, and immunity of this was carried out to it. A lymphocyte is taken out from the spleen of the mouse which carried out immunity, and it suspends in E-RDF of 10mL after centrifugal separation (200-400xg, 5 minutes), and is about

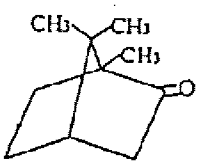
1x108. The lymphocyte of an individual was obtained. This suspension and 2x107 Cell fusion was performed promptly after preparing the myeloma suspension of an individual. This cell suspension is cultivated by a HAT medium etc. by the well of 96 hole microplate. the well which the hybridoma increased -- enzyme-labeling immunoadsorption (it Assay(s) Enzyme-Linked ImmunoSorbent [ ] --) After performing screening by the ELISA method and publication below, the serum free medium containing an antibody After centrifugal separation and filtration, The fractions containing an antibody were collected, with the tris (Tris)-hydrochloric-acid buffer solution (pH7.4) of 10mM(s), it freeze-dried, the anti-2-MIB antibody was obtained after dialysis, and cryopreservation was performed at -20 degrees C.

[0021] ** . -- the singularity trial of an anti-2-MIB antibody, and result: -- the specific monoclonal antibody was chosen as 2-MIB by measuring the reactivity by the contention ELISA method using serum-free culture supernatant liquid by using 2-MIB and analogue other than this as an isolation antigen about the obtained anti-2-MIB antibody. Since the anti-2-MIB antibody produced camphor and BSA complex as an antigen, it shows singularity to both camphor and BSA. For this reason, camphor and Ova complex were used for the fixed antigen in the exam. using this complex -- camphor or 2-MIB -- screening of a specific antibody is attained. First, as a fixed antigen, the coat of camphor and the Ova complex was carried out to the EIA plate used by the enzyme immunoassay (Enzyme Immunoassay) of 96 holes, and it was blocked. The dilution train (1000-0.01microg/mL) was made from 10% ethanol-twin (ethanol-Tween) 20-PBS for seven sorts of compounds (Camphor) similar to 2-MIB which shows a chemical formula below to coincidence, i.e., camphor, a camphor quinone (Camphorquinone), a NORUKAN fur (Norcamphor), a borneol (Borneol), isoborneol (Isoborneol), norbornane (Norbornane), and norborneol (Norborneol) 10 times. Equivalent mixing of the serum free medium containing these and each antibody was carried out, and it incubated for 30 minutes. It has checked that had almost no reactivity to norborneol, a NORUKAN fur, and norbornane, therefore

the anti-2-MIB antibody by this invention had very high singularity to 2-MIB when a singularity trial is performed for this reaction mixture as an antibody sample.

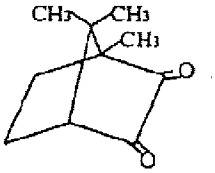
[0022]

[Formula 3]

camphor 

[0023]

[Formula 4]

camphorquinone 

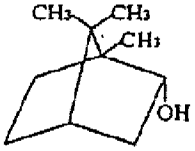
[0024]

[Formula 5]

norcamhor 

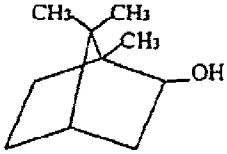
[0025]

[Formula 6]

borneol 

[0026]

[Formula 7]

isoborneol 

[0027]

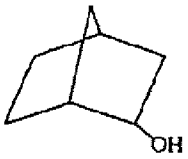
[Formula 8]

norbornane

[0028]

[Formula 9]

norborneol



[0029] ** production [of the detection equipment of quartz-resonator use 2-MIB, and the sensor for 2-MIB detection]: -- detection equipment [of 1 quartz-resonator use 2-MIB]: -- drawing 1 is the block diagram of the detection equipment of 2-MIB using frequency measurement of a quartz resonator. The sensor 1 for 2-MIB detection which attached the golden electrode to the quartz resonator, fixed camphor and an Ova complex antigen, and was blocked by BSA into the non-covered part of a golden electrode can measure weight as change of a frequency, if it connects with an oscillator circuit 2, a frequency counter 3, and a personal computer 4 and 2-MIB adheres to camphor and an Ova complex antigen. In order to set the 2-MIB detection sensor 1 into the measurement cel 5 of capacity 150mL and to maintain a fixed measurement environment, the air through activated carbon 6 is sent at a rate for 120mL(s)/by the air pump.

[0030] The sensor 1 for 2-MIB detection is produced by the approach of 23 described below.

2) Immobilization of the camphor and the Ova complex to the electrode top of a quartz resonator : drawing 2 is the flow Fig. showing the sensor for 2-MIB detection, and its production approach. Each drawing shows each matter typically. First, as an object for the dippings of a quartz resonator 11 which attached the golden electrode 12, the upper part of the micro tube for 2mL was cut, and the container of about 500microL capacity was prepared. The camphor

and the Ova complex solution of concentration 0.5 mg/mL were prepared there, the dipping of the quartz resonator 11 which attached the golden electrode 12 at the temperature of 25 degrees C for 17 hours was carried out, and camphor and the Ova complex 13 were fixed on the golden electrode 12 like drawing 2 (b). Furthermore, pure water performed washing for 35 minutes for non-fixed matter removal, the frequency was measured with the detection equipment of 2-MIB of drawing 1 after 1-hour desiccation, and fixed camphor and Ova complex weight were computed from the frequency change from initial value.

[0031] 2) Blocking by BSA of camphor and Ova complex : the dipping of the quartz resonator 14 with a golden electrode which fixed camphor and Ova complex was carried out at the temperature of 25 degrees C, and 55% of humidity for 17 hours, and blocking processing was performed for the non-covered part 15 of the electrode of a quartz resonator in the BSA solution of concentration 0.5 mg/mL by BSA16 like drawing 2 (c). Furthermore, the frequency was measured with pure water with the detection equipment of 2-MIB of after 55-minute washing and 1-hour desiccation and drawing 1 , and fixed BSA weight was found by frequency change from initial value.

[0032] Through the above-mentioned process, on the golden electrode 12 of a quartz resonator 11, camphor and the Ova complex 13 were fixed and the sensor 1 for 2-MIB detection was produced. Next, how to detect 2-MIB using this sensor is explained.

** . -- method [of detecting 2-MIB using a competitive reaction]: -- drawing 3 shows the conceptual diagram of the method of detecting 2-MIB using a competitive reaction.

[0033] First, known concentration CM The mixed solution of the anti-2-MIB antibody 18 of 2-MIB17 and the known weight WA (= known concentration CA x volume V) was made to react. The dipping of the above-mentioned sensor 1 for 2-MIB detection was carried out to this reaction solution, it put at the temperature of 30 degrees C, and the competitive reaction was performed. Next, pure water washed and the frequency was measured with the detection equipment of 2-MIB

of drawing 1 after 1-hour desiccation. Weight WB of the anti-2-MIB antibody 19 which the variation of a frequency understood from this measurement and early measurement, and was combined with the camphor and the Ova complex 13 on the sensor 1 for 2-MIB detection It asks. this result to known concentration CM Weight WC of 2-MIB17 and the anti-2-MIB antibody which reacted WC =WA-WB ****** -- it asks.

[0034] ** . -- creation [of the calibration curve by 2-MIB of known concentration]: -- the competitive reaction produced from the frequency measurement result of a quartz resonator to drawing 4 -- known concentration CM Weight WC of the anti-2-MIB antibody combined with 2-MIB Relation was shown. Anti-2-MIB antibody concentration CA in a solution It is 0.04 mg/mL regularity. It is the known 2-MIB concentration CM so that clearly from drawing 4 . The amount WC of anti-2-MIB antibodies combined with 2-MIB by changing with 0.005 - 5 mg/L It turns out that it changes proportionally. a basis [calibration curve / this to / this] -- the amount WC of joint antibodies in an anti-2-MIB antibody from -- it turned out that it is possible to carry out the quantum of the strange 2-MIB concentration.

[0035] [Example 2] The case where a transducer is a surface acoustic element (SAW component) is explained below with reference to drawing 5 and 6 about the gestalt of operation of the 2-MIB detecting method of this invention. Drawing 5 is the circuit diagram having shown the system of measurement of 2-MIB using a SAW component. As shown in this drawing, a SAW component consists of the piezo-electric substrate 21, the metal thin film 22, a propagation side 23 of surface acoustic waves, amplifier 24, and a mixer 25. Using the same approach as an example 1, camphor and Ova complex were fixed on the propagation side 23 of a SAW component (for example, 160MHz), and the competitive reaction was performed on an example 1 and these conditions.

[0036] Relation with the amount of anti-2-MIB antibodies combined with the known 2-MIB concentration and 2-MIB by frequency measurement of the SAW component obtained as a result is shown in drawing 6 . By the same result as drawing 4 using a quartz resonator, this drawing was understood that it is

possible to carry out the quantum of the strange 2-MIB concentration from the amount of joint antibodies based on this calibration curve. Furthermore, it can guess easily that a quantum can be carried out about odorant other than 2-MIB, for example, JIEOSUMIN, using the same approach.

[0037] In addition, it does not pass over numerical conditions, such as an amount of the material of construction used in the above two examples, the processing time, and processing temperature, to an example, therefore this invention is not limited to these conditions.

[0038]

[Effect of the Invention] According to this invention, 2-MIB of the strange concentration which carries out the dipping of the transducer which fixed camphor and protein complex to the solution measured [fixed concentration ****], and is contained [transducer] in a measured solution in an anti-2-MIB antibody, and the camphor and the protein complex on a transducer are made to react with an anti-2-MIB antibody competitively, and the amount of antibodies combined with the complex on a transducer is calculated by output change of a transducer. By producing the calibration curve by known 2-MIB concentration and the known amount of joint antibodies, the quantum of the amount of strangeness 2-MIB in a measured solution can be carried out. Since this reaction uses the antigen-antibody reaction, as compared with the conventional quantum approach, 2-MIB of 1 low concentration does not have the need for concentration, either, and can detect it to high sensitivity. Moreover, 2-MIB can be detected alternatively and analysis actuation of two or more data based on statistics processing is not needed with the singularity of two antibodies. 3 [moreover,] -- it has the advantage of ** which can carry out a quantum by easy actuation.

[Translation done.]

* NOTICES *

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The block diagram of the detection equipment of 2-MIB using frequency measurement of a quartz resonator

[Drawing 2] The flow Fig. showing the sensor for 2-MIB detection, and its production approach

[Drawing 3] The conceptual diagram of the method of detecting 2-MIB using a competitive reaction

[Drawing 4] A related Fig. with the amount of anti-2-MIB antibodies combined with the known 2-MIB concentration and 2-MIB by frequency measurement of a quartz resonator

[Drawing 5] The circuit diagram having shown the system of measurement of 2-MIB using a SAW component

[Drawing 6] A related Fig. with the amount of anti-2-MIB antibodies combined with the known 2-MIB concentration and 2-MIB by frequency measurement of a SAW component

[Description of Notations]

1: The sensor for dimethyl isoborneol (2-MIB) detection

2: Oscillator circuit

3: Frequency counter

4: Personal computer

5: Measurement cel

6: Activated carbon

7: Air pump

11: Quartz resonator

12: Golden electrode

13: Camphor ovalbumin (Ova) complex

14: Complex fixed quartz resonator

15: The non-covered part of a golden electrode

16: Cow serum albumin (BSA)

17:2-MIB

18: Anti-2-MIB antibody

19: A joint antibody with camphor and Ova complex

21: A piezo-electric substrate

22: Metal thin film

23: Propagation side

24: Amplifier

25: Mixer

[Translation done.]

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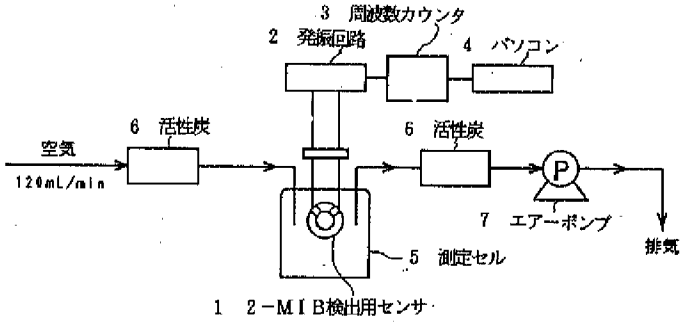
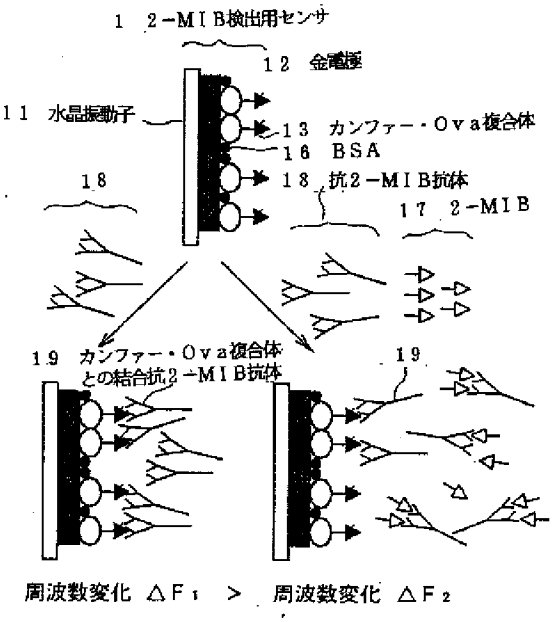
DRAWINGS

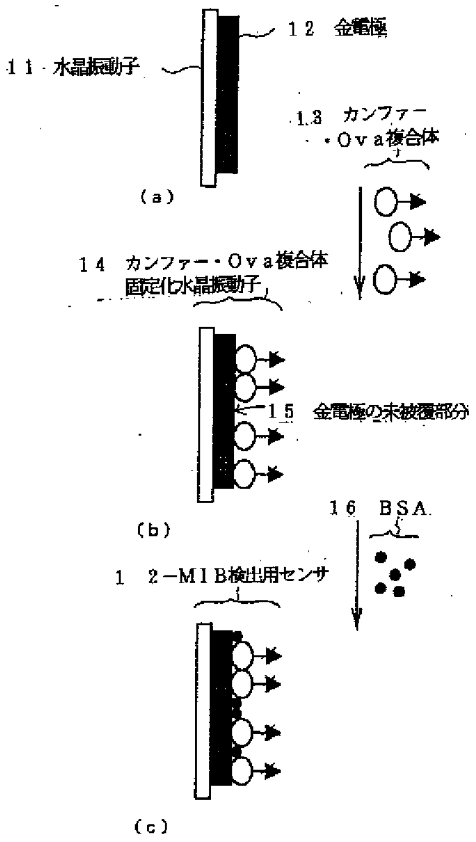
[Drawing 1]



[Drawing 3]



[Drawing 2]



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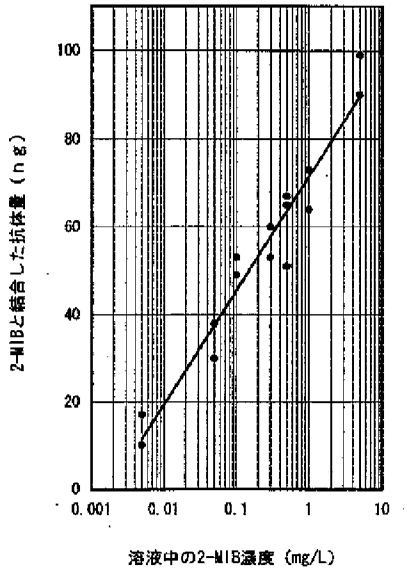

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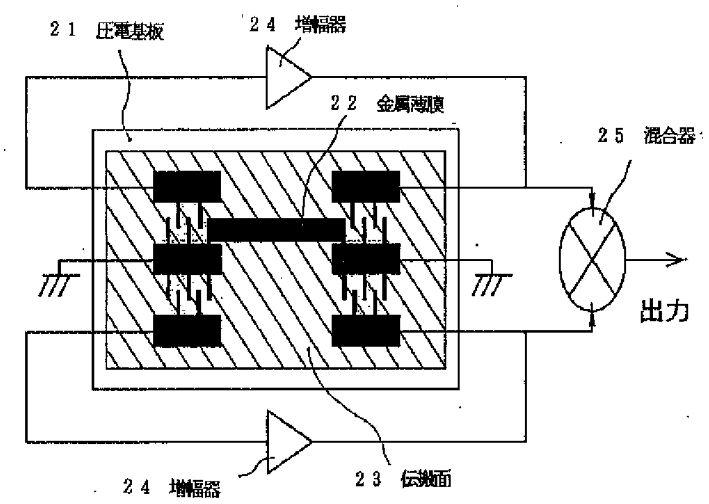

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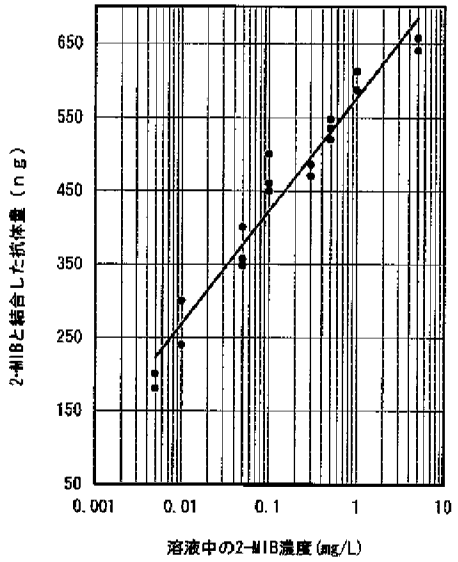

[Translation done.]

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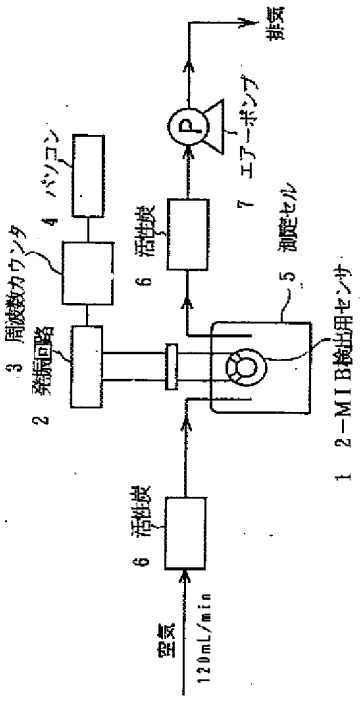
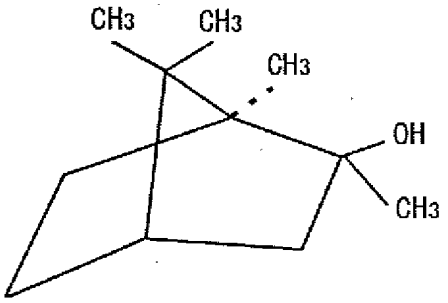
最終頁に続く

(54)【発明の名称】　２－メチルイソボルネオールの検出方法

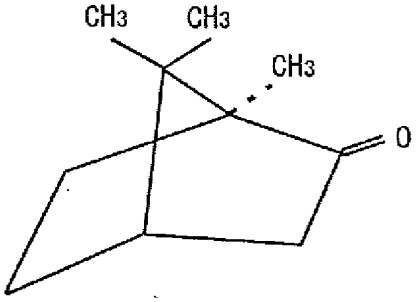
(57)【要約】
【課題】パージ・トラップＧＣ質量分析法および固相抽出ＧＣ／ＭＳ法は高精度の測定ができるが、装置が高価、長測定時間、正確な分析には熟練が必要等の問題点があり、短時間で容易に高感度に２－メチルイソボルネオール（２－ＭＩＢ）を検出できる測定法の開発が要望されていた。
【解決手段】２－ＭＩＢに構造の類似したカンファー・蛋白質複合体を固定化したトランスデューサを、抗２－ＭＩＢ抗体が一定濃度となるように調製した被測定溶液中に浸せきし、被測定溶液中に含まれる未知濃度の２－ＭＩＢと、水晶振動子または弾性表面波素子を使ったトランスデューサ上に固定化したカンファー・蛋白質複合体とを、競合的に抗２－ＭＩＢ抗体と反応させ、結果的にカンファー・蛋白質複合体と結合した抗２－ＭＩＢ抗体によるトランスデューサの出力変化により、被測定溶液中の２－ＭＩＢ濃度を定量する方法を発明した。



【特許請求の範囲】
【請求項１】２－メチルイソボルネオール（以下２－ＭＩＢと記載）に構造の類似したカンファーと蛋白質の複合体を使って作製した抗２－ＭＩＢ抗体と、測定対象の２－ＭＩＢとカンファー・蛋白質複合体抗原との、抗原抗体反応を利用した２－ＭＩＢの検出方法。
【請求項２】２－ＭＩＢに構造の類似したカンファー・蛋白質複合体抗原を固定化したトランスデューサを、抗２－ＭＩＢ抗体が一定濃度となるように調製した被測定溶液中に浸せきし、被測定溶液中に含まれる未知濃度の２－ＭＩＢと、トランスデューサ上に固定化したカンファー・蛋白質複合体抗原とを、競合的に抗２－ＭＩＢ抗体と反応させ、結果的にカンファー・蛋白質複合体抗原と結合した抗２－ＭＩＢ抗体によるトランスデューサの出力変化により、被測定溶液中の２－ＭＩＢ濃度を定量する２－ＭＩＢの検出方法。
【請求項３】請求項１および２に記載の抗２－ＭＩＢ抗体は、カンファーと牛血清アルブミンの複合体を用いて作製することを特徴とする２－ＭＩＢの検出方法。
【請求項４】請求項１および２に記載のカンファー・蛋白質複合体抗原は、蛋白質が卵白アルブミンであることを特徴とする２－ＭＩＢの検出方法。
【請求項５】請求項２に記載のトランスデューサは、水晶振動子または弾性表面波素子（ＳＡＷ素子）であることを特徴とする２－ＭＩＢの検出方法。
【発明の詳細な説明】
【０００１】
【発明の属する技術分野】本発明は、水中に含まれるカビ臭物質である２－ＭＩＢを免疫反応により検出する方法に関する。
【０００２】
【従来の技術】水道の異臭味についての苦情や被害は年々増加の傾向にある。この異臭味は、水源の汚染や湖沼などの富栄養化による藻類の大量発生で生成される、２－メチルイソボルネオール（２－ｍｅｔｙｌｉｓｏｂｏｒｎｅｏｌ：２－ＭＩＢ）やジェオスミンなどのカビ臭物質が原因である。このため、近年、水道水に対して安全でおいしい水の供給への要望が国民の間で高まってきており、この要望を受けて、より質の高い水道水の安定供給を目指すために、水道水質基準を補完する項目としてカビ臭物質を含む「快適水質基準項目」が設定されている。この基準達成のためには、においにかかわるセンサや高度浄水処理システムの開発が重要である。
【０００３】現在、浄水の水質管理では、人間が鼻で一定時間ごとににおいを嗅いで監視を行っている。また、カビ臭の分析手段としては、公定法としてパージ・トラップガスクロマトグラフ質量分析法及び固相抽出ＧＣ／ＭＳ法が採用されている。しかしこれらの分析手段は、水質の常時監視という観点からは幾つかの問題点があり、簡便かつ迅速で個人差のない測定方法や装置の開発が要望されている。
【０００４】一方、食品工業や化学薬品の品質管理分野では、においについての測定器として、人間の鼻に近い閾値を持つ『においセンサ』が開発され、数社から市販されている。代表的な商品の測定原理には、におい物質を、１）抵抗値の変化で検出する金属酸化物半導体センサ、２）伝導率変化で検出するコンダクティングポリマー、３）重量変化で検出する脂質被覆水晶振動子を用いたセンサなどがある。
【０００５】また、研究開発段階では、カビ臭物質の発光遺伝子による定量法なども報告されているが（石橋良信ほか、土木学会第50回年次学術講演会講演概要集、ｐ１３０２～１３０３、１９９５）、定量可能な２－ＭＩＢの最低濃度は０．１ｍｇ／Ｌである。
【０００６】
【発明が解決しようとする課題】上記の公定法は、高精度の測定を行うことができるが、１）装置が高価である、２）試料の濃縮も含め１回の測定時間が約５時間と長い、３）操作が煩雑で正確に分析するには熟練を要するため、ルーチン分析には難しい面がある、などの問題点がある。
【０００７】また、市販のにおいセンサについては、１）感度不足や人間の感覚との相関性に欠ける、２）あるにおいに対して数種のセンサ応答のパターン比較によりにおいの判別を行うため、特定のにおいに対する選択性がなく、統計処理による計測データの解析を必要とする、３）定量測定が困難である。従って、市販のにおいセンサを浄水の臭気物質監視へ適用するためには、感度、選択性、定量性など問題を解決する必要がある、などの問題がある。
【０００８】この発明は、上記の問題点を解決し、短時間に容易にそして高感度に２－ＭＩＢを検出することが可能な測定法を提供することを目的としている。
【０００９】
【課題を解決するための手段】この目的を達成するため、本発明では、カビ臭原因物質である２－ＭＩＢと類似の構造を有するカンファー（Ｃａｍｐｈｏｒ）を利用し、特異性（高選択性）と高感度を抗原抗体反応により達成する。
【００１０】
【化１】



【００１１】
【化２】



【００１２】具体的には、上記のカビ臭原因物質である２－ＭＩＢと類似の構造を有するカンファーと蛋白質の複合体抗原を固定化したトランスデューサを、２－ＭＩＢに特異的（選択的）に結合する抗２－ＭＩＢ抗体を一定濃度含む被測定溶液中に浸せきし、溶液中に存在している未知濃度の２－ＭＩＢとこのカンファー・蛋白質複合体抗原とを競合的に反応させ、結果的にトランスデューサ上に固定化したカンファー・蛋白質複合体抗原に結合した抗２－ＭＩＢ抗体量をトランスデューサの出力変化により求め、２－ＭＩＢが存在した場合の結合抗体量からの差分として、被測定溶液中の２－ＭＩＢ濃度を定量することを特徴とする。

【００１３】また、本発明は、抗２－ＭＩＢ抗体を作製するにあたり、カンファーと牛血清アルブミン（Ｂｏｖｉｎｅ　Ｓｅｒｕｍ　Ａｌｂｕｍｉｎ：以下ＢＳＡと記載）の複合体（以下カンファー・ＢＳＡ複合体と記載）を抗原とすることを特徴とする。従って、１）水に難溶で扱いにくい２－ＭＩＢに代わり水溶性のカンファーを用いることで抗体の作製が容易になる、２）カンファーは低分子であり単独では抗原性を示さないため、ＢＳＡと結合させることで抗原性をもたせることが可能になる、という利点が生じる。

【００１４】また、２－ＭＩＢの競合反応に用いる複合体をカンファーと卵白アルブミン（Ｏｖａｌｂｕｍｉｎ：以下Ｏｖａと記載）の複合体（以下カンファー・Ｏｖａ複合体と記載）することによって、カンファー・ＢＳＡ複合体を抗原として作製した抗２－ＭＩＢ抗体が、カンファー・Ｏｖａ複合体との反応の際、カンファーのみを認識して結合することができる。

【００１５】また、本発明は、トランスデューサに水晶振動子または弾性表面波素子（ＳＡＷ素子）を用いて、反応前後の周波数化から抗２－ＭＩＢ抗体の結合量を重量変化として検出し、上述の方法で２－ＭＩＢを定量できる。

【００１６】
【発明の実施の形態】
［実施例１］以下、この発明でトランスデューサが水晶振動子（ＡＴカット、１０ＭＨｚ）の場合について、①トランスデューサに固定化するカンファー・Ｏｖａ複合体の作製、②被測定溶液中に入れる抗２－ＭＩＢ抗体の作製、③抗２－ＭＩＢ抗体の特異性試験と結果、④水晶振動子利用２－ＭＩＢの検出装置と２－ＭＩＢ検出用センサの作製、⑤競合反応を利用した２－ＭＩＢの検出法、⑥既知濃度の２－ＭＩＢによる検量線の作成、の順に説明する。

【００１７】①．トランスデューサに固定化するカンファー・Ｏｖａ複合体の作製：カンファー１ｇとオルソカルボキシメトキシルアミン・ヘミ（Ｏ－Ｃａｒｂｏｘｙｍｅｔｈｏｘｙｌａｍｉｎｅ　ｈｅｍｉ）塩酸塩２．８４ｇに１４ｍＬのエタノールと１６．５ｍＬの２規定の水酸化ナトリウムを加え、６時間還流し、２５℃で一晩放置した。これに２００ｍＬの純水を加え、２ｍｏｌ／ｍ^3 の水酸化ナトリウムを用いてｐＨ９．５に調製し、これを３０ｍＬの酢酸エチルで３回抽出後、水層を１規定の塩酸でｐＨ３に調製した。これを０℃で一晩静置し、生じた沈殿を遠心分離（３０００回転／分で５分）によって集め、５００ｍＬの純水で洗浄後、硫酸カルシウムの存在下で減圧乾燥することでカンファーカルボキシメチルオキシム（Ｃａｍｐｈｏｒ－ｃａｒｂｏｘｙｍｅｔｈｙｌｏｘｉｍｅ、以下ＣＭＯと記載）を得た。

【００１８】次に１８．９ｍｇのＣＭＯと１２μＬのトリエチルアミン（Ｔｒｉｅｔｈｙｌａｍｉｎｅ）に１ｍＬのテトラヒドロフラン（ｔｅｔｒａｈｙｄｒｏｆｕｒａｎ）を加えて－５℃で冷却後、１２μＬのイソブチルクロロフォルメート（Ｉｓｏｂｕｔｙｌ　ｃｈｌｏｒｏｆｏｒｍａｔｅ）を加えて－５℃で３０分間振とうした。この反応液を冷却した２０ｍｇ／ｍＬのＯｖａ溶液５ｍＬ中に滴下し、４℃で一晩振とうした。これを純水で透析し、カンファー・Ｏｖａ複合体を得た。保存は、－２０℃で凍結保存を行った。

【００１９】②．被測定溶液中に入れる抗２－ＭＩＢ抗体の作製：抗原としての２－ＭＩＢは水に難溶で取り扱いが困難であるため、抗体の作製には２－ＭＩＢに類似の構造を有するカンファーを利用した。ただし、カンファーは低分子量で抗原性をもたないため、蛋白質などの高分子を結合させて抗原とすることが必要である。この蛋白質としては、強い抗原性をもつＯｖａより、比較的抗原性の弱いＢＳＡが適当である。そこで、①に述べた

カンファー・Ｏｖａ複合体と同様の方法でカンファー・ＢＳＡ複合体抗原を作製した。

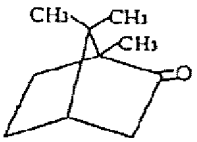
【００２０】次に、抗２－ＭＩＢ抗体の作製には本抗原をＰＢＳ（Ｐｈｏｓｐｈａｔｅ　Ｂｕｆｆｅｒｅｄ　Ｓａｌｉｎｅ）で希釈し、この希釈液３００μＬと「ナカライテスク（株）」製のフロイト完全アジュバンド（Ｆｒｅｕｎｄ　Ｃｏｍｐｌｅｔｅ　Ａｊｕｖａｎｔ）の３００μＬを用いて免疫用のエマルジョンを作製した。これを５週令オスの「成和実験動物」ＢＡＬＢ／Ｃマウスに腹腔内注射を行い免疫した。免疫したマウスの脾臓からリンパ球を取り出し、遠心分離後（２００～４００×ｇ、５分）１０mＬのＥ－ＲＤＦに懸濁して、約1×10^{8} 個のリンパ球を得た。この懸濁液と2×10^{7} 個のミエローマ懸濁液を調製後、速やかに細胞融合を行った。この細胞懸濁液を９６穴マイクロプレートのウェルでＨＡＴ培地などで培養し、ハイブリドーマが増殖したウェルについては酵素標識免疫吸着法（Ｅｎｚｙｍｅ－Ｌｉｎｋｅｄ　ＩｍｍｕｎｏＳｏｒｂｅｎｔ　Ａｓｓａｙ、以下ＥＬＩＳＡ法と記載）によるスクリーニングを行った後、抗体を含む無血清培地を遠心分離とろ過後、抗体を含む画分を回収して１０mＭのトリス（Ｔｒｉｓ）－塩酸緩衝液（ｐＨ７．４）で透析後、凍結乾燥して抗２－ＭＩＢ抗体を得て、－２０℃で凍結保存を行った。

【００２１】③．抗２－ＭＩＢ抗体の特異性試験と結果：得られた抗２－ＭＩＢ抗体について無血清培養上清を用い、２－ＭＩＢとこれ以外の類似化合物を遊離抗原として競合ＥＬＩＳＡ法による反応性を測定することにより、２－ＭＩＢに特異的なモノクローナル抗体の選択を行った。抗２－ＭＩＢ抗体はカンファー・ＢＳＡ複合体を抗原として作製したため、カンファーとＢＳＡの両方に対して特異性を示す。このため、本試験では、固定化抗原にはカンファー・Ｏｖａ複合体を用いた。この複合体を用いることで、カンファーまたは２－ＭＩＢ特異的な抗体のスクリーニングが可能となる。まず、固定化抗原としてカンファー・Ｏｖａ複合体を９６穴の酵素免疫定量法（Ｅｎｚｙｍｅ　Ｉｍｍｕｎｏａｓｓａｙ）で使用するＥＩＡプレートにコートし、ブロッキングした。同時に、化学式を以下に示す２－ＭＩＢと類似の７種の化合物、即ち、カンファー（Ｃａｍｐｈｏｒ）、カンファーキノン（Ｃａｍｐｈｏｒｑｕｉｎｏｎｅ）、ノルカンファー（Ｎｏｒｃａｍｐｈｏｒ）、ボルネオール（Ｂｏｒｎｅｏｌ）、イソボルネオール（Ｉｓｏｂｏｒｎｅｏｌ）、ノルボルナン（Ｎｏｒｂｏｒｎａｎｅ）、ノルボルネオール（Ｎｏｒｂｏｒｎｅｏｌ）を１０％エタノール－ツイン（ｅｔｈａｎｏｌ－Ｔｗｅｅｎ）２０－ＰＢＳで１０倍希釈列（１０００～０．０１μｇ／ｍＬ）を作った。これらとそれぞれの抗体を含む無血清培地を等量混合し、３０分間インキュベートした。この反応液を抗体サンプルとして特異性試験を行ったところ、ノルボルネオール、ノルカンファー、ノルボルナンに対してほとんど反応性を持たず、従って本発明による抗２－ＭＩＢ抗体は、２－ＭＩＢに対し非常に高い特異性を持つことが確認できた。

【００２２】

【化３】

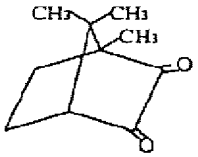
camphor



【００２３】

【化４】

camphorquinone



【００２４】

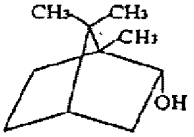
【化５】

norcamhor

【００２５】

【化６】

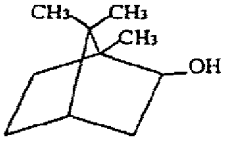
borneol



【００２６】

【化７】

isoborneol



【００２７】

【化８】

norbornane

【００２８】

【化９】

norborneol

【００２９】④　水晶振動子利用２－ＭＩＢの検出装置と２－ＭＩＢ検出用センサの作製：
１）水晶振動子利用２－ＭＩＢの検出装置：図１は水晶振動子の周波数測定を利用した２－ＭＩＢの検出装置のブロック図である。水晶振動子に金電極をつけ、カンファー・Ｏｖａ複合体抗原を固定化し金電極の未被覆部分にＢＳＡでブロッキングした２－ＭＩＢ検出用センサ１は、発振回路２、周波数カウンタ３、パソコン４と接続され、カンファー・Ｏｖａ複合体抗原に２－ＭＩＢが付着すると、周波数の変化として重量が測定できる。２－ＭＩＢ検出センサ１は容量１５０ｍＬの測定セル５の中にセットされ、一定の測定環境を維持するために、活性炭６を通した空気をエアポンプにより１２０ｍＬ／分の割合で送られている。
【００３０】２－ＭＩＢ検出用センサ１は次に述べる２）３）の方法で作製される。
２）水晶振動子の電極上へのカンファー・Ｏｖａ複合体の固定化：図２は、２－ＭＩＢ検出用センサおよびその作製方法を示すフロー図である。各図では、それぞれの物質を模式的に示している。まず、金電極１２をつけた水晶振動子１１の浸せき用として、２ｍＬ用マイクロチューブの上部を切断して約５００μＬ容量の容器を準備した。そこへ濃度０．５ｍｇ／ｍＬのカンファー・Ｏｖａ複合体溶液を調製し、温度２５℃で１７時間、金電極１２をつけた水晶振動子１１を浸せきし、図２（ｂ）のように金電極１２上にカンファー・Ｏｖａ複合体１３を固定化した。さらに、未固定化物質除去のために純水で３５分洗浄を行い、１時間乾燥後、図１の２－ＭＩＢの検出装置により周波数を測定し、初期値からの周波数変化から固定化カンファー・Ｏｖａ複合体重量を算出した。
【００３１】２）カンファー・Ｏｖａ複合体のＢＳＡによるブロッキング：濃度０．５ｍｇ／ｍＬのＢＳＡ溶液に、温度２５℃，湿度５５％で１７時間、カンファー・Ｏｖａ複合体を固定化した金電極付き水晶振動子１４を浸せきし、図２（ｃ）のように水晶振動子の電極の未被覆部分１５をＢＳＡ１６でブロッキング処理を行った。さらに、純水で５５分洗浄，１時間乾燥後、図１の２－ＭＩＢの検出装置により周波数を測定し初期値からの周波数変化により、固定化ＢＳＡ重量を求めた。
【００３２】上記の工程を経て、水晶振動子１１の金電極１２の上にカンファー・Ｏｖａ複合体１３を固定化して２－ＭＩＢ検出用センサ１を作製した。次に、本センサを用いて２－ＭＩＢを検出する方法について説明する。
⑤．競合反応を利用した２－ＭＩＢの検出法：図３は競合反応を利用した２－ＭＩＢの検出法の概念図を示す。
【００３３】まず、既知濃度C_M の２－ＭＩＢ１７と既知重量W_A （＝既知濃度C_A ×体積Ｖ）の抗２－ＭＩＢ抗体１８の混合溶液を反応させた。この反応溶液に上述の２－ＭＩＢ検出用センサ１を浸せきし、温度３０℃で静置して競合反応を行った。次に純水で洗浄し１時間乾燥後、図１の２－ＭＩＢの検出装置により周波数を測定した。この測定と初期の測定とから周波数の変化量が分かり、２－ＭＩＢ検出用センサ１上のカンファー・Ｏｖａ複合体１３と結合した抗２－ＭＩＢ抗体１９の重量W_B が求められる。この結果から既知濃度C_M の２－ＭＩＢ１７と反応した抗２－ＭＩＢ抗体の重量W_C は$W_C = W_A - W_B$ として求められる。
【００３４】⑥．既知濃度の２－ＭＩＢによる検量線の作成：図４には、水晶振動子の周波数測定結果から作製した、競合反応によって既知濃度C_M の２－ＭＩＢと結合した抗２－ＭＩＢ抗体の重量W_C との関係を示した。溶液中の抗２－ＭＩＢ抗体濃度C_A は０．０４ｍｇ／ｍＬ一定である。図４から明らかな様に、既知２－ＭＩＢ濃度C_M を０．００５～５ｍｇ／Ｌと変えることによって、２－ＭＩＢと結合した抗２－ＭＩＢ抗体量W_C が比例して変化していくことが分かる。このことから、この検量線をもとに抗２－ＭＩＢ抗体中の結合抗体量W_C から未知の２－ＭＩＢ濃度を定量することが可能であることが分かった。
【００３５】［実施例２］次にこの発明の２－ＭＩＢ検出法の実施の形態についてトランスデューサが弾性表面波素子（ＳＡＷ素子）である場合について図５、６を参照して説明する。図５はＳＡＷ素子を利用した２－ＭＩＢの測定系を示した回路図である。この図に示すようにＳＡＷ素子は圧電基板２１、金属薄膜２２、表面弾性波の伝搬面２３、増幅器２４、混合器２５から構成される。実施例１と同様の方法を用いて、ＳＡＷ素子（例えば１６０ＭＨｚ）の伝搬面２３上にカンファー・Ｏｖａ複合体を固定化し、実施例１と同条件で競合反応を行った。
【００３６】その結果得られたＳＡＷ素子の周波数測定による既知２－ＭＩＢ濃度と２－ＭＩＢと結合した抗２－ＭＩＢ抗体量との関係を図６に示す。この図は水晶振動子を利用した図４と同様の結果で、この検量線をもとに結合抗体量から未知の２－ＭＩＢ濃度を定量することが可能であることが分かった。さらに、２－ＭＩＢ以外の臭気物質、例えばジェオスミンについても同様の方法を用いて定量できることは容易に類推できる。
【００３７】なお、以上の２つの実施例で用いた使用材料の量、処理時間、処理温度などの数値的条件は一例にすぎず、従ってこの発明は、これら条件に限定されるものではない。
【００３８】

【発明の効果】この発明によれば、カンファー・蛋白質複合体を固定化したトランスデューサを抗２－ＭＩＢ抗体を一定濃度含む被測定溶液に浸せきし、被測定溶液中に含まれる未知濃度の２－ＭＩＢと，トランスデューサ上のカンファー・蛋白質複合体とを競合的に抗２－ＭＩＢ抗体と反応させ、トランスデューサ上の複合体に結合した抗体量をトランスデューサの出力変化により求める。既知の２－ＭＩＢ濃度と結合抗体量による検量線を作製しておくことで、被測定溶液中の未知２－ＭＩＢ量を定量することができる。この反応は、抗原抗体反応を利用しているため、従来の定量方法と比較して、１）低濃度の２－ＭＩＢでも濃縮の必要がなく高感度に検出できる。また、２）抗体の特異性により、２－ＭＩＢを選択的に検出でき、統計処理による複数データの解析操作がいらない。また、３）容易な操作で定量することができる、等の長所を有する。

【図面の簡単な説明】

【図１】水晶振動子の周波数測定を利用した２－ＭＩＢの検出装置のブロック図

【図２】２－ＭＩＢ検出用センサおよびその作製方法を示すフロー図

【図３】競合反応を利用した２－ＭＩＢの検出法の概念図

【図４】水晶振動子の周波数測定による既知２－ＭＩＢ濃度と２－ＭＩＢと結合した抗２－ＭＩＢ抗体量との関係図

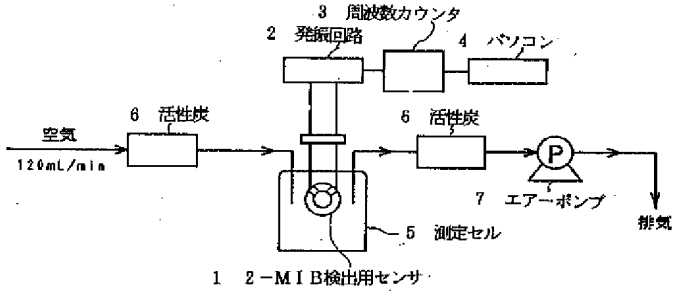
【図５】ＳＡＷ素子を利用した２－ＭＩＢの測定系を示した回路図

【図６】ＳＡＷ素子の周波数測定による既知２－ＭＩＢ濃度と２－ＭＩＢと結合した抗２－ＭＩＢ抗体量との関係図

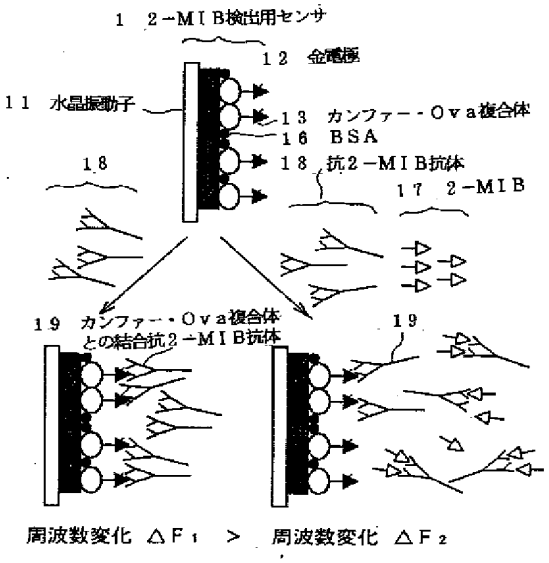
【符号の説明】

１：ジメチルイソボルネオール（２－ＭＩＢ）検出用センサ
２：発振回路
３：周波数カウンタ
４：パソコン
５：測定セル
６：活性炭
７：エアーポンプ
１１：水晶振動子
１２：金電極
１３：カンファー・オボアルブミン（Ｏｖａ）複合体
１４：複合体固定化水晶振動子
１５：金電極の未被覆部分
１６：牛血清アルブミン（ＢＳＡ）
１７：２－ＭＩＢ
１８：抗２－ＭＩＢ抗体
１９：カンファー・Ｏｖａ複合体との結合抗体
２１：圧電基板
２２：金属薄膜
２３：伝搬面
２４：増幅器
２５：混合器

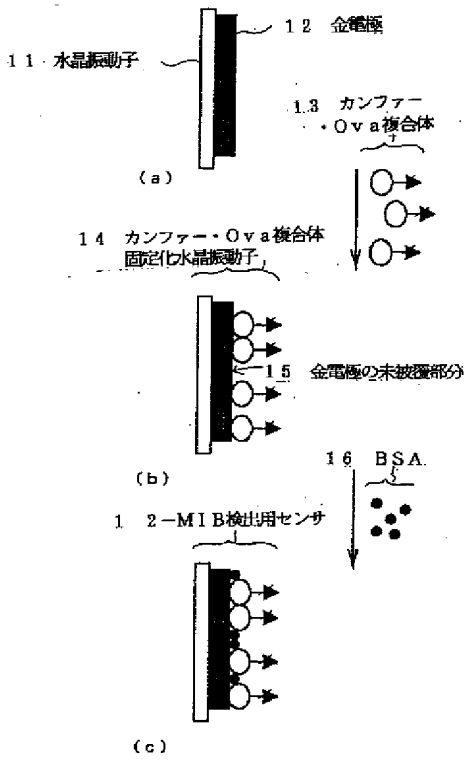
【図１】



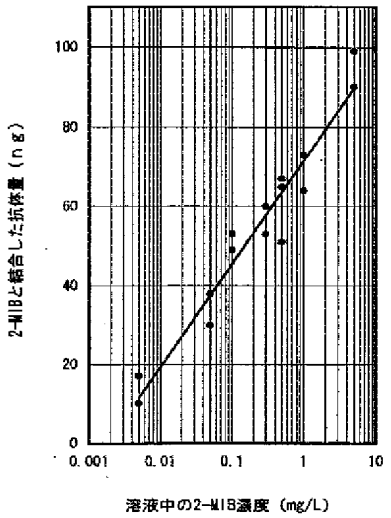
【図３】



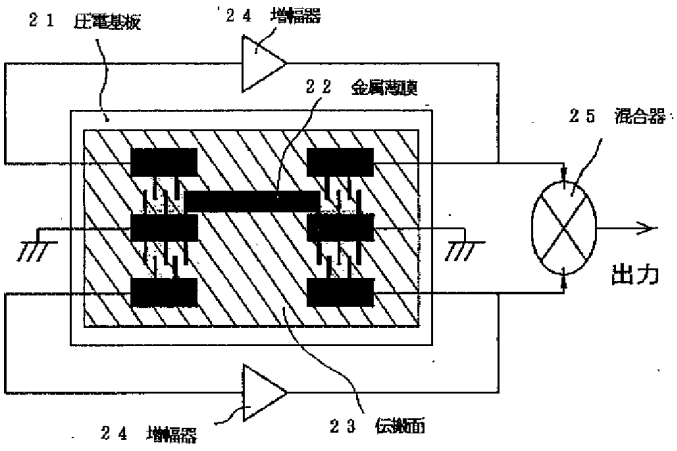
【図２】



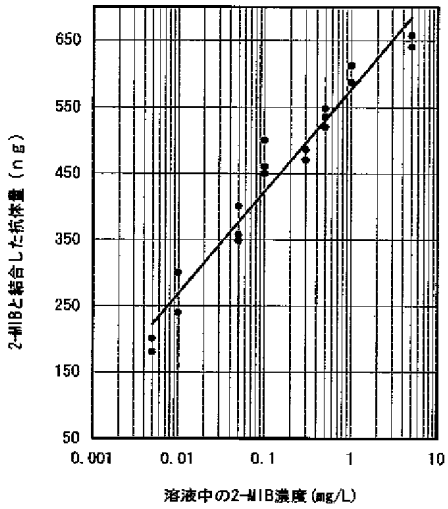
【図４】



【図５】



【図６】



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